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CALCULATION OF HEMOGLOBIN, HEMOTOCRIT AND PLASMA PROTEIN
(THE COPPER SULPHATE METHOD)

1. Knowledge of the blood hemoglobin, hematocrit and plasma protein values is a prerequisite to the most intelligent use of various parenteral fluids.

2. These values can be obtained readily by the use of the copper sulphate method and it is recommended that laboratories of all hospitals be provided with the necessary equipment and properly trained personnel to make determinations. Inclosure 1 is a chart from which calculations are made, as described in Inclosure 2 which gives all technical information necessary.

3. General and station hospitals should requisition the necessary supplies and prepare their own sets. Either the sixteen or thirty two bottle set is suitable for the usual clinical studies. The following should be requisitioned:

a. Item #4056400 Bottle, screw neck, vial type, round, 120 c.c.

b. Item #1161500 Cupric sulphate, 1 lb; U.S.P.

Four pounds of copper sulphate are required for an adequate stock of standard solution of specific gravity 1.100 and from which all other solutions are prepared as described in Inclosure 2.

4. Field, evacuation hospitals and clearing companies should requisition only Item #4056400 in par. 3a. and they will be supplied stock copper sulphate solution from the army or base laboratory. From the stock solution sixteen bottle sets are easily prepared as described in Inclosure 2.

[signature]
GUY B. DENIT
Brigadier General, USA,
Chief Surgeon

2 Incls:
Incl 1 - Chart.
Incl 2 - Details of the method.

THE COPPER SULFATE METHOD FOR MEASURING SPECIFIC GRAVITIES OF WHOLE BLOOD AND PLASMA

This method makes it possible with three or four drops of blood and no apparatus except a medicine dropper and small bottles of copper sulfate solution to determine the specific gravity of the blood, and from it the hemoglobin content within 10 percent. By examining in a like manner the serum or plasma from the same blood one can determine also the plasma protein concentration and increase the accuracy of the hemoglobin estimation to ± 2 percent. To measure the blood and plasma gravities and calculate the plasma proteins, hemoglobin and hematocrit on a line chart requires about 2 minutes.

The results can be used as follows:

1. To assist in ascertaining the results of hemorrhage.

2. To estimate decrease in plasma volume from hemoglobin increases, and to decide whether the plasma volume decrease is due to loss of water (dehydration of cholera, dysentery, exposure), or to loss also of plasma proteins (extravasation in burns, trauma, etc.).

3. To assist in deciding whether blood replacement therapy requires administration of saline solution or plasma or whole blood.

4. To follow the result of such therapy and decide whether it has been adequate, and when it must be repeated.

5. If the number of cases exceeds the amount of blood and plasma available, the method will assist in deciding which cases must receive it and which may be able to do without.

6. Besides the acute conditions, the method will assist in diagnosing the different types of anemia, and in detecting various pathological conditions partailly summarized in a later section, in which the plasma proteins become diluted or concentrated.

The utility of blood and plasma concentration measurements is well recognized in the diagnosis and treatment of shock from wounds, burns, hemorrhage, etc., and in other conditions in which plasma proteins or hemoglobin are affected. However, the gravity methods hitherto used which are of sufficient accuracy, such as the gravimetric, the falling drop method and the gradient tube require precision instruments on stable bases. None of these methods, for example, could well be used on shipboard.

Principle of the method: The technic consists of letting drops of plasma or whole blood fall into a graded series of solutions of copper sulfate of known specific gravity, and noting whether the drops rise or fall in the solutions. Each drop on entering the solution becomes encases in a sack of copper-proteinate, and remains as a discrete drop without change of gravity for 15 or 20 seconds, during which its rise or fall reveals its gravity relative to

Incl 2.

Principle of the method (Cont.).

that of the solution. The size of the drop does not have to be constant, hence no special pipette is needed for delivering the drops. No temperature correction is needed, because the temperature coefficient of expansion of the copper sulfate solutions approximates that of the blood plasma. This method is capable of measuring gravities to ± 0.00005, which is ten times the accuracy required. The copper sulfate solution automatically cleans itself after each test, because within a minute or two after the test is completed the material of the drop settles to the bottom as a precipitate.

The principle of dropping blood into a series of solutions of known gravity has been applied for decades but has never achieved entire success. Mixtures of organic liquids have been used, e.g., benzene and chloroform, but they were liable to change in specific gravity due to differential evaporation of the components. They also have temperature coefficients of expansion several times that of water or blood and cannot be used without accurate temperature regulations. In addition, some of them give rise to toxic and to explosive vapours. Dispersion of blood occurs too rapidly in the usual aqueous salt or glycerol solutions to enable small gravity differences, to be measured easily and accurately. The use of aqueous standard solutions which have protein-coagulating power overcomes these difficulties.

For accurate work, viz., gravities within ± 0.0002, a series of copper sulfate solutions graded at intervals of 0.001 in specific gravity is used; twenty solutions cover the plasma range 1.015 -1.035 and forty cover the whole blood range, 1.035 - 1.075. For rougher work with gravities accurate to ± 0.001, sixteen solutions at intervals of 0.004 suffice to cover the entire range of blood and plasma.

Procedure for Gravity Analysis of Blood and Plasma: The drop of serum, plasma or whole blood is delivered from a height of about 1 cm. above the solution from a pipette, or from a needle attached to a glass syringe. It is preferable to use small drops for the reason that they permit more tests before the standard solution must be changed. Therefore a pipette with a fine tip is preferable to a coarse one for delivering the drop. Greasing the side of the tip with vaseline also reduces the size of the drop, especially if the vaseline is mixed with a little caprylic alcohol.

The delivered drop breaks through the surface film of the solution and penetrates 2 to 3 cms. below the surface; within 5 seconds the momentum of the fall is lost and the drop then either begins to rise, or becomes stationary, or continues to fall. The gravity of the drop relative to the solution does not change appreciably until the drop has been immersed in the solution for about 15 seconds, and there is ample time to note its behavior during this interval. If the drop is lighter than the test solution it will rise, perhaps only a few millimeters during this interval, and may begin to sink immediately afterwards. If the drop is of the same gravity as the standard test solution it will become stationary for this interval and then fall. If the drop is heavier it will continue to fall during the interval. In summary, the behavior during the 10 seconds after the drop has lost the momentum of its fall into the solution indicates whether the drop is lighter or heavier than the test solution; if it rises at all during this period it is lighter than the standard.

Example: The following example shows how, by bracketing on the probable extremes of a plasma's gravity range and then testing intermediate points, one can find the correct gravity with not more than 4 drops to within ± 0.0002.

The plasma was expected to be of normal or greater concentration. Four successive drops gave the following results, in which the figures indicate the gravities of the standards, and + or - indicates that the plasma was heavier or lighter than the standard; 1.027 +; 1.031 -; 1.029 +; 1.030 -. The plasma was heavier than 1.029 and lighter than 1.030, and could therefore be placed 1.0295 with an error less than ± 0.0002.

Approximate Field Determinations: For field work it may suffice to determine the gravities to ± 0.001. For this only 16 standard solutions with gravity intervals of 0.004 covering the range from 1.016 to 1.076 will be needed. An error of 0.001 in plasma gravity affects plasma proteins by 0.3 gram per 100cc; additive errors of 0.001 in the gravities of both plasma and whole blood affect hemoglobin results by 5 percent.

Example: The plasma tested was lighter than 1.028 and heavier than 1.024. By observing the behavior of the drop in the two solutions it was noted to be closer to 1.028 than to 1.024 and hence could be placed at 1.027 with an error not greater than ± 0.001.

Calculations: Line charts for the conversion of plasma and whole blood gravities to plasma protein concentration, hemoglobin concentration and hematocrit percentages have been prepared by standard methods, and are given in Inclosure 1.

The calculations are made by laying a straight edge or stretched thread as directed on the charts.

If anticoagulant other than heparin is used the correction indicated at the bottom of the line chart, Fig. 2 should be applied.

For men the normal values are taken from precise measurements made for this purpose on the blood and plasma of 20 normal men. For women normal hemoglobin and cell volume are taken as 90 percent of the normal for men.

Precautions:

1. A tourniquet applied for more than 1 minute will cause measurable hemoconcentration.

2. Anticoagulants.

(a) Heparin, 0.2mgm. per cc. blood, does not change gravities.

(b) Oxalate mixture, 1 mgm. per cc. blood, increases gravities of both plasma and whole blood about 0.0004. This effect is negligible for most purposes; it raises calculated plasma protein and hemoglobin values each by only 0.1 gm. per 11 cc. When, however, less than 4 cc. of blood is added to a tube containing 5 mgm. of oxalate mixture, corrections are made by subtracting from both the whole blood and the plasma gravities 0.0007 if the blood volume is 3 cc., 0.0010 if it is 2 cc., 0.0020 if it is 1cc.

(c) Citrate introduces large errors.

3. Analysis without Anticoagulants. Anticoagulants can be dispensed with if the gravity of the whole blood is determined by dropping it directly from the syringe needle into the standard copper sulfate solutions. The remainder of the blood can be transferred to a tube and permitted to clot. From the centrifuged or sedimented material a few drops of serum are drawn up into a dropper and are used for serum gravity determination.

4. Renewing standards. Renewal is needed when about 1 small drop of blood or plasma per cc. of standard solution has been added. This will decrease the gravity of the solution by 0.0005. A 4 ounce bottle of standard serves for about 100 tests. One extra standard of gravity 1.027 is prepared and to the solution one-fortieth of its volume of plasma is added dropwise. When the volume of precipitate in the bottom of a repeatedly used standard equals that in this control bottle, the standard is renewed.

5. Surface film. Occasionally a drop will fail to make a clean break through the surface film of the copper sulfate solution, and remain attached by a tentacle to the film. In this case the drop is detached from the film by tapping the tube, and a fresh drop is tried.

After each test one makes sure that the surface film is left clean and free from fragments. If any are left on the film they are likely to prevent a clean break-through of the drop in the next test. Fragments caught in the surface film can usually be detached by tapping the tube; they then sink to the bottom. Sometimes, however, a fragment of fatty nature holding a bubble will continue to float on the surface. Such fragments are removed with a wooden applicator stick.

6. Temperature. The method requires but little attention to temperature.

(a) Change of temperature of standard solutions of $\pm$ 10°C, changes gravities not more than $\pm$ 0.0002, because the temperature effect is nearly the same on the gravities of standards as of blood.

(b) Blood drawn from a vein into a syringe can be delivered directly from the syringe needle into standard solutions at 20° or above without error exceeding 0.0003 in the gravity measurement. Blood or plasma drawn into a medicine dropper and delivered into the copper sulfate solutions should be within 5° Centigrade of the temperature of the solutions.

(c) Convection currents in the standard solutions could introduce false readings. Do not bring cold bottles into a warm room and use at once. Do not leave bottles near stove, or window sill, etc. Hold bottles only by top when using, not by sides.

Bottles of Tubes for the Standard Copper Sulfate Solutions: For precise determination (gravities to ± 0.0002) 70 "oval prescription" bottles of 4, 2, or 1-ounce capacity. For field determination (gravities to ± 0.001) 16 bottles suffice.

Rubber stoppers, corks, or screw-tops are preferable to glass stoppers. The bottles are labeled with gravity figures from 1.008 to 1.075, with the labels where they can be read from above, and are arranged in ranks of five, for convenience in selecting desired solutions. Seventy 4-ounce bottles thus arranged occupy a space about 10 x 20 inches.

For laboratory use the 4-ounce bottles are preferable because they permit about 100 analyses, without replacing the solution.

For portable sets the 1 or 2 ounce bottles may be used. It is convenient to bind those in sets of 5 with transparent "Scotch tape".

Test tubes of 16 x 150 mm. size, or preferable 25 x 200, heavy-walled, can be used in place of bottles.

Apparatus for Preparing the Copper Sulfate Solutions: One volumetric flask of 100cc. capacity and one burette, preferably also of 100cc capacity, for preparing standard copper sulfate solutions to be stored in 4-ounce bottles. If 2-ounce bottles are used the flask and burette should be of 50 cc. capacity; If 1-ounce bottles are used the flask and burette should be of 25 cc. capacity.

Three 4-liter bottles.

One 1-liter volumetric flask.

One 500 cc. graduated cylinder.

One 7-inch funnel, and cotton or filter paper.

One thermometer, 0°C. - 40°C, or corresponding Fahrenheit.

Reagents: Oxalate mixture. 3 gm. ammonium oxalate and 2 gm. potassium oxalate are dissolved in 250cc. H_2O. 0.25cc. are pipetted into round-bottom, heavy-walled, 15 x 125 mm., pyrex tubes. The tubes are placed on their sides, to spread solution in a film, in an incubator (not over 50°C.) and dried. Mark the outside of the tubes at the 5cc. level with a glass-marking pencil.

The oxalate mixture (Hellor and Paul, J.Lab.Clin.Med., 19, 777 (1934), disturbs cell and plasma gravities less than either potassium or ammonium oxalate alone.

Crystalline copper sulfate, $CuSO_4,5H_2O$. This is preferably purchased in the form of "fine crystals." Otherwise it must be pulverized before using. Four pounds provide a complete set of 100cc. standard solutions. Ten pounds will probably suffice a laboratory for a year.

Preparation of Copper Sulfate Solutions: Saturated copper sulfate solution - This solution is used to prepare a stock solution of gravity 1.100 ± 0.0003. Use of a solution saturated at a known temperature affords a precise means of preparing the stock solution without a balance. The saturated solution is prepared as follows:

Four pounds of "fine crystals," or pulverized copper sulfate are placed in a 4-liter bottle. About 2500cc. of distilled water is added, and the bottle is stoppered and shaken vigorously for a total of 5 minutes, which need not be continuous. (Three minutes has been found sufficient, even at 0°C., to saturate this solution if the sulfate is well pulverized.) As soon as the shaking is finished the temperature of the solution is taken to the nearest half degree Centigrade and is recorded. (It will be a little cooler than the water was before the saturation, because the saturation process absorbs heat.) The solution is immediately decanted off the crystals and is filtered, to remove fine suspended crystals, through cotton or dry filter paper into a clean, dry 4-liter bottle. The solution is at once used to make up a stock solution of gravity 1.100. (It is preferable not to let the saturated solution stand long before using, as if it cools, some of the copper sulfate may crystallize and change the concentration.) The undissolved sulfate can be used again.

Two and a half liters of the saturated solution suffice for more than 4 liters of the stock solution of gravity 1.100, and this in turn is sufficient for a complete set of 70 standard solutions of 100cc. volume each, with enough surplus to provide replacements for the standards which are most used. Smaller or larger amounts of the saturated solution can be made by using proportional amounts of copper sulfate and water.

Stock Copper Sulfate Solution of Gravity 1.100. (Gravity figures in this report were determined as the ratio of the weight of copper sulfate solution to the weight of an equal volume of water at the same temperature.) The volume of saturated solution indicated in Table 1 is measured in a 500cc. graduated cylinder and poured into a 1-liter volumetric flask. The upturned cylinder is allowed to drain into the flask for 30 seconds. The flask is then filled to the mark with water, and is inverted several times to mix the solution. The mixing results in a contraction, so that the meniscus now falls below the mark. The flask is let stand for a minute until the solution drains down from the neck. Then enough additional water is added to bring the volume to 1 liter, the solution is mixed, and then poured into a clean, dry, 4-liter bottle. The same 1-liter volumetric flask is used to prepare 3 more liters of the stock copper sulfate solution of gravity 1.100. Each time before the flask is used again it is rinsed with water and the rinsings are discarded.

It is desirable that the saturated solution, the stock solution and the standard solutions next described shall all be prepared at within 5° Centigrade of the same temperature. The coefficients of expansion of the saturated and stock copper sulfate solutions are slightly but definitely greater than that of water, so that if the saturated solution and stock solution were prepared at 35°C. and the standard solutions at 20°C. the standards would have more copper sulfate than intended, enough to increase the gravity by about 0.001.

Preparation of Standard Solutions in 100cc. Portions. The standard solutions are prepared in 100cc. portions when 4-ounce bottles are available for storage.

For the standard of 1.075 gravity, 74cc. of stock solution of gravity 1.100 are measured from the burette into the 100cc flask, the flask is filled to the mark with water, and the solution is mixed and transferred to a labeled 4-ounce bottle, which is stoppered to prevent evaporation.

To prepare the standard of gravity 1.074, the 100cc. flask is rinsed once with water and the burette is refilled from a 250cc. Erlenmeyer flask containing the solution. The 73cc. of the stock solution are measured into the volumetric flask and diluted to 100.

The same procedure is carried through for preparation of the entire series down to 1.015, which covers the extreme ranges for blood and plasma. If gravities on ascitic fluid and transudates may be desired also, the series is extended to 1.008. For each standard the number of cc. of stock solution less by 1 than the number indicated in the second and third decimal places of the desired gravity is measured in the rinsed 100cc. flask and diluted to the mark.

If there were no contraction when the stock solution is mixed with water one would dilute 75cc of the stock to 100cc. to get a gravity of 1.075 etc. However, there is a contraction which is empirically corrected by taking 1cc. less of the stock. It happens conveniently that the same 1cc. correction serves for the entire range, 1.075 to 1.008, over which its use yields gravities correct within $\pm$ 0.0003.

For field work with gravities accurate to $\pm$ 0.001, sixteen standard solutions varying by 0.004 and covering the range of 1.016 -1.076 are prepared.

Preparation of Standard Solutions in 50 or 25cc. Portions. The volumes of stock solutions indicated in Table 2 are measured from 50 or 25 cc. burettes into 50 or 25 cc. flasks.

Equations on Which the Line Charts are Based:

G_p = Specific gravity of plasma; G_B = Specific gravity of whole blood.

1.0970 = average specific gravity of normal cells.

(1) Plasma protein (gm. per 100 cc. plasma) = 343 G_p -1.0070

(2) Hematocrit (cc. cells in 100cc whole blood) = 100 $\dfrac{G_B - G_p}{1.0970 - G_p}$

(3) Oxygen capacity (cc. O_2 bound in 100cc. whole blood) = 46.1 $\dfrac{G_B - G_p}{1.0970 - G_p}$

(4) Hemoglobin (grams per 100cc. whole blood) = 33.9 $\dfrac{G_B - G_p}{1.097 - G_p}$

46.1 represents mean cc. of oxygen bound by hemoglobin in 100 cc. of cells. 33.9 indicates the mean grams of hemoglobin in 100 cc. of cells.

The constants 1.0970, 46.1 and 33.9 were found by precise oxygen capacity, hematocrit, and gravity determinations on the blood of 20 normal men.

The above equations can be used to calculate results in case the line charts are not available.

Effects of Cell Abnormalties on Hematocrits and Hemoglobins Calculated from Gravities: In blood cells of abnormal hemoglobin content, as in hypochromic anemias, the calculations of hematocrits and hemoglobins from gravity values is not as accurate as in blood with cells of normal composition. However, we have seldom encountered a blood in which the error in hemoglobin was greater than 1 volume per cent O_2 capacity, or 0.7 gm. of hemoglobin per 100cc.

The reason why even marked abnormalties in the hemoglobin content of the cells do not cause greater errors in blood hemoglobin concentrations calculated from gravities is presumably the fact that changes in cell gravity and cell hemoglobin contents are parallel. The calculations (Equations 2, 3, and 4) assume that the cell gravity remains constant at 1.0970 and the hemoglobin content at 33.9 grams per 100 cc. of cells. When both these values change in the same direction their changes in Equation 4 partly cancel each other in their effects on hemoglobin calculated from gravity values. The hematocrit values calculated from gravities in hypochromic anemia may be less accurate.

Pathological Conditions Affecting the Plasma Protein Concentration: The different types of anemia and polycythemia are so well known in their effects on blood hemoglobin concentration that it is unnecessary to discuss them here. However, the numerous conditions that cause a decrease or increase in the plasma protein concentration are not so well known. The most adequate sumary of them is the recent one by Kagan (Southern Med. J., Birmingham, Alabama, 36, 234 (1943) and Table 3 is drawn chiefly from his data.

So far as the clinical utility of plasma protein determination is concerned, one may say that in acute conditions the determinations may serve chiefly as guides in therapy to correct abnormal volume or protein concentration of the plasma, while in chronic conditions the determinations serve more as aids in diagnosis of pathological conditions.

In diagnosis, an abnormal plasma protein concentration is definite proof that one of the factors controlling the concentration has been disturbed.

However, a normal protein concentration is not final proof that the factors controlling the concentration are all working normally, for there may be abnormalities with opposite effects which balance; in such a case the plasma gravity fails to indicate the pathological conditions that nevertheless exist. Such a balance may occur in either an acute condition, such as hemorrhage, or a chronic one, such as liver cirrhosis or diabetes.

Hemorrhage at first causes a general hemodilution, affecting both plasma proteins and hemoglobin as interstital fluid enter the circulation from the tissue spaces to replace the lost blood. If the hemorrhage leads to shock, however, hemoconcentration may set in, with decrease of blood volume and return of the plasma and hemoglobin concentrations towards or even above normal.

Liver cirrhosis retards albumin synthesis, but may increase globulin formation, with a resultant normal total protein concentration in the plasma.

Chronic diabetes tends to produce malnutrition and deficit of plasma albumin and total protein, but in acidosis, dehydration may raise the total protein concentration to normal or above.

It is evident from the above that, although blood and plasma concentrations data provide information concerning a patient's condition that can not be obtained by clinical inspection alone, nevertheless the concentration data can not be applied by any rule of thumb, but must be considered together with the history and other available data in deciding on the patient's condition and the indicated therapy.

Burns, Post-Operative Conditions, and Traumatic Shock: In these three conditions, with their rapid and often critical shifts of hemoconcentration, estimations of the plasma proteins and the hemoglobin by the gravity technic are of especial utility, both in detecting the changes and in guiding the therapy. For data on which the following summary is chiefly based the authors are indebted to Dr. John Scudder (Personal communication).

Burns. Seepage of plasma from the denuded areas cause a decrease both in the volume and the protein concentration of the plasma. In consequence the plasma protein falls while the hemoglobin rises.

In some cases during the first hours a loss of water from the blood occurs to such an extent that the plasma proteins show a transitory rise. This, however, is followed by the fall, described above, as the effects of protein loss by seepage accumulate. The occassional initial dehydration of the blood seems to be due partly to passage of water from blood to tissues, as it may occur when there is no marked external loss of fluid, as by vomiting and sweating. The hemoglobin rises during this stage, as well as during the subsequent stage when the effects of seepage dominate.

To guide plasma replacement therapy it is desirable to determine plasma and blood gravities one or more times daily for several days.

Post-Operative conditions. In the post-operative period dehydration of the blood is likely to increase both the plasma protein and the hemoglobin concentrations. The dehydration may go so far as to produce uremia. Repeated saline injections may be required to replace fluid. However, if too much saline is administered by unregulated infusions, hemodilution and a water-logged, edematous state of the tissues may result which is as undesirable as the dehydration.

If changes in blood gravity are followed infusions can be so regulated that error in either direction is avoided.

For repeated injections it is safer to use Hartmann's (J. Am.Med.Asso., 103, 1349 (1934)) solution rather than 0.9 percent NaCl. Hartmann's solution contains 6 gm. NaCl, 4 gm. sodium lactato, 0.4 gm. KCl, 0.2gm. CaCl alone may result in fall of plasma bicarbonate, potassium, and calcium. The loss of bicarbonate is particularly undesirable, because it exacerbates the acidosis that may be produced by either shock or anesthesia. The lactate in Hartmann's solution is equivalent to 75 percent as much bicarbonate, because it is burned to bicarbonate in the body.

Besides their use in directing fluid replacement therapy, blood gravity measurements can assist in detecting certain post-operative complications. Peritonitis, intestinal fistulae, abscesses, and pancreatitis all cause the same blood changes soon after burns, viz., fall in plasma proteins and rise in hemoglobin. (In pancreatitis an initial period of increased plasma protein may intervene, as in some cases of burns). In perforations of the gastro-intestinal tract the plasma proteins first rise, then fall. The behavior of the hemoglobin depends on the extent of hemorrhage, the variable effects of which have already been discussed. A fall in hemoglobin indicates marked loss of blood, but maintenance of hemoglobin concentrations is not certain evidence against such loss.

Traumatic shock. Varying degrees of dehydration, of plasma protein seepage from injured vessels, tissues, and surfaces and of internal and external hemorrhage, can combine to produce such unpredictable effects on blood volume and concentration that observations on the blood are specially needed, together with careful interpretation. Marked changes in hemoglobin or plasma protein concentration provide clear guidance. But it is possible for blood dehydration so to balance losses of plasma and hemoglobin that their concentrations remain, or return to normal. Such a paradoxical condition is to be suspected when the clinical and vascular signs of shock accompany normal gravity values. The circulating volumes of blood is then low, despite the normal concentrations, and transfusion of whole blood in preference to plasma is indicated.

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(From the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research, Commanding Officer, Commander Thomas M. Rivers.

BY: Robert A. Phippils,* Donald D. Van Slyke,* Vincent P. Dole,*
Kendall Emerson, Jr.,* Paul B. Hamilton, and Reginald M. Archibald.
With the technical assistance of Emily G. Stanley and John Plazin.

*Lt, (MC) U.S.N.R.

TABLE 1

(Copper Sulf. Method for Sp.Gr. of Blood and Plasma, Phillips, et al.)

Cc of Saturated Copper Sulfate Solution to be diluted to 1-liter to Give the Stock Solution of Specific Gravity 1.100

Temperature in °C. or °F. refers to the temperature of the saturated solution at the time of saturation (end of shaking for 5 minutes).

Cc. = cc. of the saturated copper sulfate solution to be diluted to 1000cc to give the stock solution of specific gravity 1.100.

Temperature °C.	Temperature °F.	cc.	Temperature °C	Temperature °F.	cc.	Temperature °C.	Temperature °F.	cc.
10.0	50.0	578	20.0	68.0	488	30.0	86.0	425
10.5	50.9	573	20.5	68.9	484	30.5	86.9	423
11.0	51.8	568	21.0	69.8	480	31.0	87.8	420
11.5	52.7	563	21.5	70.7	477	31.5	88.7	417
12.0	53.6	558	22.0	71.6	473	32.0	89.6	414
12.5	54.5	553	22.5	72.5	469	32.5	90.5	412
13.0	55.4	548	23.0	73.4	466	33.0	91.4	409
13.5	56.3	543	23.5	74.3	463	33.5	92.3	406
14.0	57.2	539	24.0	75.2	460	34.0	93.2	403
14.5	58.1	534	24.5	76.1	456	34.5	94.1	401
15.0	59.0	529	25.0	77.0	453	35.0	95.0	398
15.5	59.9	525	25.5	77.9	450	35.5	95.9	395
16.0	60.8	521	26.0	78.8	447	36.0	96.8	392
16.5	61.7	516	26.5	79.7	445	36.5	97.7	390
17.0	62.6	512	27.0	80.6	442	37.0	98.6	387
17.5	63.5	508	27.5	81.5	439	37.5	99.5	384
18.0	64.4	504	28.0	82.4	436	38.0	100.4	381
18.5	65.3	500	28.5	83.3	434	38.5	101.3	379
19.0	66.2	496	29.0	84.2	431	39.0	102.2	376
19.5	67.1	492	29.5	85.1	428	39.5	103.1	373
20.0	68.0	488	30.0	86.0	425	40.0	104.0	370

TABLE 2

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Cc of Stock Copper Sulfate Solution to be Diluted to 100cc., 50cc. or 25cc. when Standard Solutions Accurate to ± 0.0001 are desired.

G	100	50	25	G	100	50	25
1.008	7.33	3.67	1.84	1.042	41.00	20.50	10.25
9	8.32	4.16	2.08	43	42.00	21.00	10.50
10	9.31	4.66	2.33	44	43.00	21.50	10.75
11	10.30	5.15	2.58	45	44.00	22.00	11.00
12	11.29	5.65	2.83	46	45.00	22.50	11.25
13	12.28	6.14	3.07	47	46.00	23.00	11.50
14	13.27	6.64	3.32	48	47.00	23.50	11.75
15	14.26	7.13	3.57	49	48.00	24.00	12.00
16	15.25	7.63	3.82	50	49.00	24.50	12.25
17	16.24	8.12	4.06	51	50.00	25.00	12.50
18	17.23	8.62	4.31	52	51.00	25.50	12.75
19	18.22	9.11	4.56	53	52.00	26.00	13.00
20	19.21	9.61	4.81	54	53.00	26.50	13.25
21	20.20	10.10	5.05	55	54.00	27.00	13.50
22	21.19	10.60	5.30	56	55.00	27.50	13.75
23	22.17	11.09	5.55	57	56.00	28.00	14.00
24	23.15	11.58	5.79	58	57.00	28.50	14.25
25	24.14	12.07	6.04	59	58.00	29.00	14.50
26	25.12	12.56	6.28	60	59.00	29.50	14.75
27	26.10	13.05	6.53	61	60.00	30.00	15.00
28	27.08	13.54	6.77	62	61.00	30.50	15.25
29	28.06	14.03	7.02	63	62.00	31.00	15.50
30	29.04	14.52	7.26	64	63.00	31.50	15.75
31	30.02	15.01	7.51	65	64.00	32.00	16.00
32	31.00	15.50	7.75	66	65.00	32.50	16.25
33	32.00	16.00	8.00	67	66.00	33.00	16.50
34	33.00	16.50	8.25	68	67.04	33.52	16.76
35	34.00	17.00	8.50	69	68.08	34.04	17.02
36	35.00	17.50	8.75	70	69.12	34.56	17.28
37	36.00	18.00	9.00	71	70.16	35.08	17.54
38	37.00	18.50	9.25	72	71.20	35.60	17.80
39	38.00	19.00	9.50	73	72.24	36.12	18.06
40	39.00	19.50	9.75	74	73.27	36.64	18.32
41	40.00	20.00	10.00	75	74.30	37.15	18.58

TABLE 3

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Conditions Causing Abnormally High or Low Concentrations of Protein in the Plasma

General Causes

High Concentrations.	Low Concentrations.
Due usually either to dehydration or to increased globulins. Increased globulins are common in chronic infections and diseases of the reticulo-endothelial system.	Due usually either to mechanical loss of proteins by extravasation or renal excretion, or to decreased albumin formation as the result of malnutrition or liver disease.

Specific Causes

High concentrations.

1. Dehydration.

a. Insufficient fluid intake, especially when accompanied by exposure, as in open boats.
b. Fluid loss: Intestinal obstruction and fistulae; diarrhea, especially in infants, also cholera and dysentery; vomiting; severe diabetic acidosis; intense heat and exertion; Addison's disease; shock, surgical and traumatic; burns, first few hours (some cases); fulminant infections.

2. Diseases involving the reticulo-endothelial system (High globulins)

Multiple myeloma.
Monocytic leukemia.
Liver cirrhosis and cancer.

3. Chronic infections. (High globulins.)

Ulcerative tuberculosis; syphilis; lymphopathia venerum; subacute bacterial endocarditis; periarteritis nodosa; lupus erythematosis; rheumatoid arthritis; Boeck's sarcoid; leprosy; kala azar; schistosomiasis; filariasis; trypanosomiasis.

Low concentrations.

1. Physical escape of plasma proteins from the circulation.

Hemorrhage, acute or chronic. weeping wounds or skin lesions (burns). Albuminuria. Shock, surgical and traumatic.

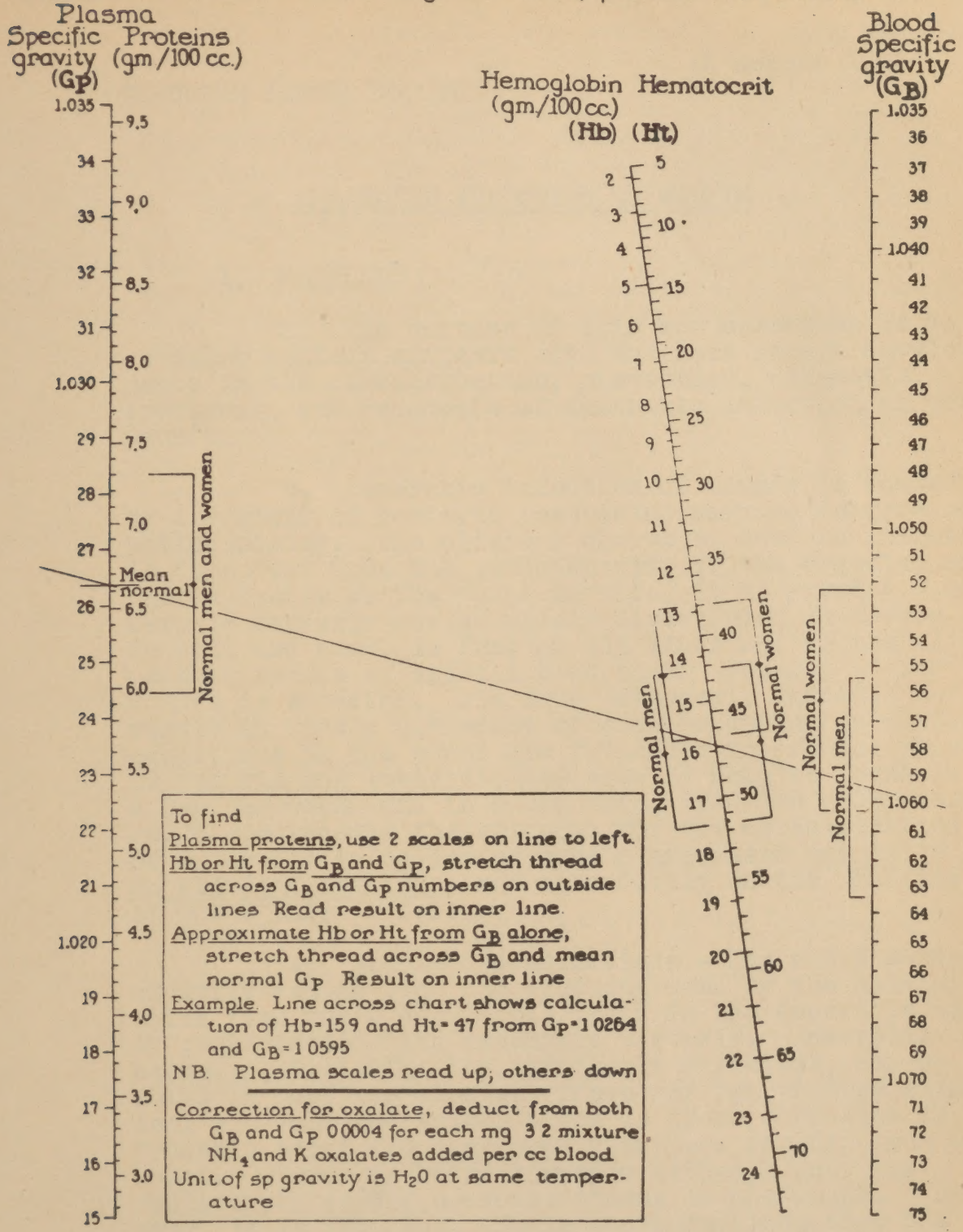
2. Malnutrition. (Low albumin.)

Low protein diet.
Vitamin deficiencies, beri-beri pellagra, etc.
Incomplete absorption, sprue.
Cancer of stomach, pancreas.
Pernicious anemia.
Diabetes mellitus, unregulated.
Hyperthyroidism
Toxemias of pregnancy.

3. Conditions in which albumin synthesis is retarded, presumably because of liver damage (Low albumin.

Cirrhosis and cancer of liver, chr. poisoning, benzene, phosphorus, etc.

Line chart for calculating plasma proteins, hemoglobin and hematocrit from gravities of plasma and blood



Incl. 1

FIGURE 2